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# Visualization of microparticle flow in the mouse brain in an intracardiac perfusion model

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## ABSTRACT

Particles with sizes in the order of a few micrometers can significantly enhance the capabilities of optoacoustic imaging systems by improving visualization of arbitrarily oriented vascular structures and achieving resolution beyond the acoustic diffraction barrier. Particle tracking may also be used for mapping the blood flow in two and three dimensions. However, a trade-off exists between the particle absorption properties and size, whereas large sized microparticles also tend to arrest in the capillary network. We analyzed the flow of microparticles in an intracardiac perfusion mouse model in which blood is effectively substituted by artificial cerebrospinal fluid (ACSF). This enables mitigating the strong blood absorption background in the optoacoustic images thus facilitating the visualization of microparticles. A sequence of three-dimensional optoacoustic images of the mouse brain is then acquired at a high frame rate of 100 Hz after injection of the particles in the left heart ventricle. By visualizing the flow of particles of different sizes in microvascular structures it is possible to establish optimal trade-offs between the particle size, their optoacoustic signal and perfusion properties.

**Keywords:** Optoacoustic Imaging, Photoacoustic Imaging, Microparticles, Localization.

## 1. INTRODUCTION

The endogenous contrast provided by blood empowers optoacoustic (OA, photoacoustic) tomography (OAT) with unique capabilities for angiographic imaging [1,2]. Blood pool contrast agents can enhance the contrast at the expense of a higher optical attenuation and lower achievable depth, hence are generally not used for rendering images of vascular structures at multiple spatial scales [3,4,5,6]. The onset of OAT systems with real-time imaging capabilities in two- and three dimensions have facilitated mapping the flow of blood pool agents, which resulted in a new capability to define perfusion-related bio-markers [7,8,9]. However, most contrast enhancement approaches used in OAT are based on active or passive targeting agents [10]. In this regard, absorbing dyes and nanoparticles smaller than 200 μm are generally used, which cannot be individually detected in the presence of a large concentration of highly absorbing red blood cells (RBCs).

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Being able to detect individual particles can significantly enhance the OAT imaging performance. For example, the fluctuations induced by flowing particles have been used to enhance the visibility under limited-view conditions and the imaging resolution in phantom experiments [11,12]. Fluctuations associated to the flow of RBCs have also been observed with transducers operating at higher frequencies, which have also been exploited for image enhancement [12,13]. Localization of individual particles further enabled forming images with a resolution significantly beyond the acoustic diffraction barrier [14,15,16], which can massively boost the OAT imaging performance. The applicability of these approaches *in vivo* relies however on the availability of sufficiently absorbing microparticles that do not cause damage to the animal e.g. by blocking the capillary network.

Herein, we make use of an intracardiac perfusion mouse model that effectively substitutes blood by artificial cerebrospinal fluid (ACSF) to visualize the flow of microparticles in the mouse brain. The aim is to extract some conclusions on the feasible sizes of solid particles so that these effectively flow in the vascular network.

# 2. METHODS

## 2.1 Intracardiac perfusion model

The intracardiac perfusion model has been recently described [17]. First, the mouse was anesthetized via intraperitoneal injection of 87.5 mg/kg Ketamine (Bremer Pharma, Wabing, Germany) and 12.5 mg/kg Xylazine (Bela-pharm, Vechta, Germany). 75 U of Heparin (Ratiofarm GbmH, Ulm, Germany) diluted in 100 ml 0.9% NaCl Solution (Braun AG, Melsungen, Germany) was additionally injected intraperitonially. A lethal dose of 87.5 mg/kg Ketamine (Bremer Pharma, Wabing, Germany) and 12.5 mg/kg Xylazine (Bela-pharm, Vechta, Germany) was then injected after placing the mouse in supine position and fixing it with a custom-made stereotactic holder (Narishige International Limited, London, UK) and needles in its extremities (Fig. 1a). The ventral portion of the rib cage was removed with an incision from the mid abdomen to the sternum and ACSF was supplied with Carbogen (Linde Group, Munich, Germany) through a 25G butterfly needle inserted in the left ventricle. The right atrium was opened with an incision to guarantee perfusion of ACSF and removal of blood. The ACSF pressure was maintained below the physiological maximum of 100 mmHg throughout the measurements. All the animal experiments were carried out in accordance with the regulations of the Helmholtz Center Munich and with approval from the Government District of Upper Bavaria.

## 2.2 Optoacoustic tomographic imaging system

OAT imaging of the mouse brain was performed with a previously described spherical array (Imasonic SaS, Voray, France) consisting of 512 piezocomposite elements densely distributed on a spherical surface with 40 mm radius and 140° angular coverage [18]. The individual elements of the array have 5 MHz central detection frequency and >80% -6dB bandwidth. An optical parametric oscillator (OPO)-based laser (Innolas GmbH, Krailling, Germany) tuned to 700 nm optical wavelength and 100 Hz pulse repetition frequency was used to illuminate the sample via a custom-made fiber bundle (Ceramoptec GmbH, Bonn, Germany) inserted in an 8 mm central aperture of the array. The volume enclosed between the mouse head and the detection surface of the array was filled with agar. The light fluence at the surface of the mouse was maintained below 20 $mJ/cm^2$. Images were reconstructed with a graphics processing unit (GPU) implementation of a back-projection formula [19].

### 2.3 Microparticle injection

Microparticles were intracardially injected in the mouse through the butterfly needle inserted in the left ventricle. Specifically, absorbing polyethylene microspheres with diameter ranges of 20-27 μm (Cospheric BKPMS 20-27um) and 10-20 μm (Cospheric BKPMS 10-20um) were suspended in phosphate buffered saline (PBS) with 3% Tween 20 surfactant. Approximately 1 ml of each suspension was injected. All the animal experiments were carried out in accordance with the regulations of the Helmholtz Center Munich and with approval from the Government District of Upper Bavaria.

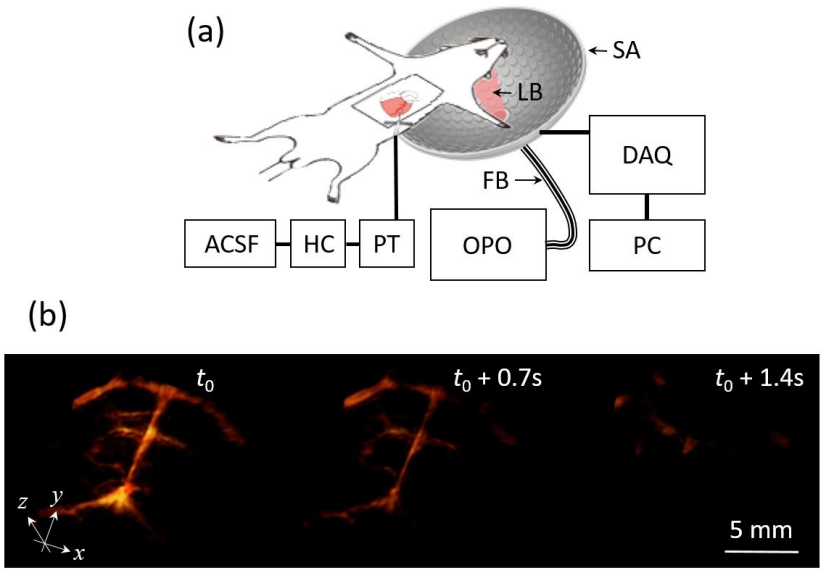


Figure 1: Intracardiac perfusion model. (a) Lay-out of the experimental set-up. ACSF – artificial cerebrospinal fluid, HC – heating coil, PT – pressure transducer, OPO – optical parametric oscillator laser, PC – personal computer, DAQ – data acquisition system, FB – fiber bundle, LB – light beam, SA – spherical array. (b) Three dimensional views of the optoacoustic images at three different time points when blood is substituted by ACSF.

## 3. RESULTS

Fig. 1b shows three-dimensional views of the OAT images of the mouse brain for 3 different instants during ACSF perfusion. It is shown that most of the blood is effectively removed from the brain. This facilitates imaging of small particles which would otherwise be arguably shadowed by the strong background absorption. Fig. 2 displays the maximum intensity projections (MIPs) along the depth direction of the OAT images for 4 different time points during the injection of 10-20 μm (top) and 20-27 μm particles (bottom). It is shown that the 20-27 μm particles could clearly be detected as they flow before being permanently arrested in the capillary network. On the other hand, the 10-20 μm could also be detected, although the signal intensity is lower. This is expected considering that the strength of the OA signal generated by a particle is in principle proportional to the total energy being absorbed in the volume of the particle. These smaller particles also arrested in the capillary network. However, the amount of observed particles in the image decreased with time after injection. This could indicate slower flow for some of the particles.

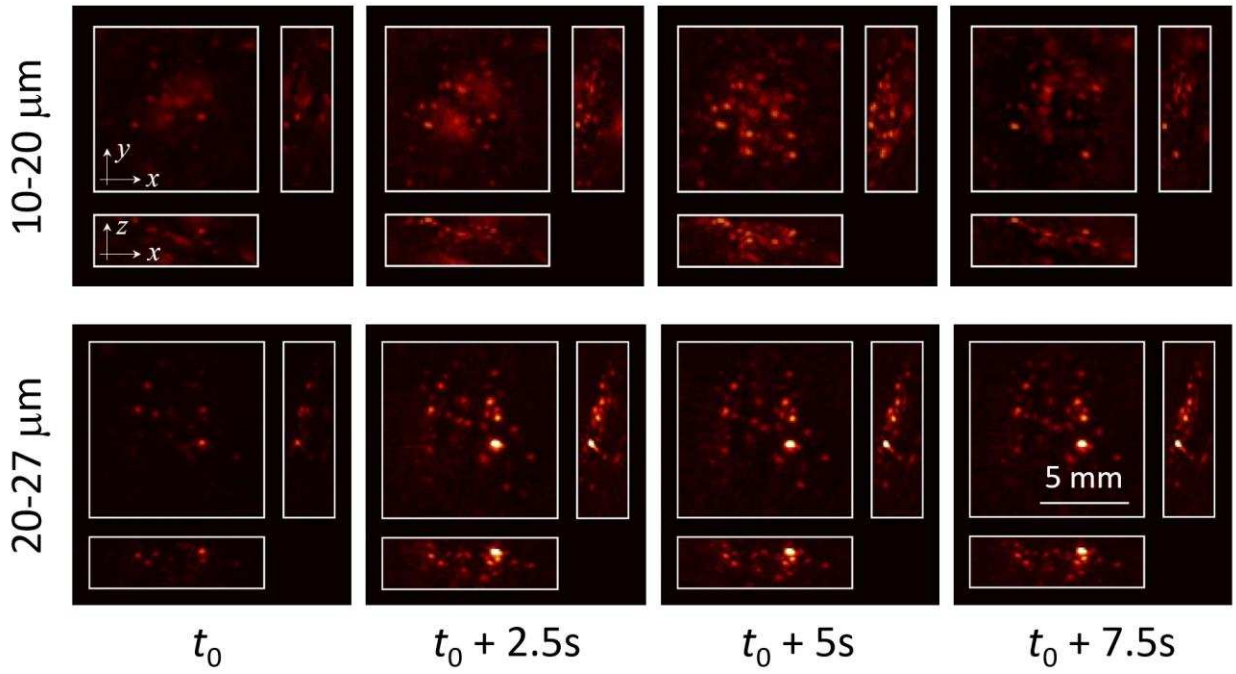


Figure 2: Visualization of microparticle flow. Maximum intensity projections (MIPs) along the *x*, *y* and *z* directions of the optoacoustic images corresponding to 4 different instants during the injection of 10-20 µm (top) and 20-27 µm particles (bottom).

## 4. CONCLUSIONS

The results presented in this work illustrate the feasibility to visualize and track microparticles in the mouse vascular network. Capillary arrest was however observed due to the large particle size. This effect was lower for the smallest microparticles, which as expected generated OA signals with less amplitude. Overall, it was shown that solid particles larger than 10 µm do not efficiently flow within vascular structures, so that smaller particles are required for the *in vivo* applicability of localization optoacoustic tomography (LOT) and other super-resolution methods based on image fluctuations induced by particle flow.

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## REFERENCES


[1] Wang, L. V., Yao, J. "A practical guide to photoacoustic tomography in the life sciences," Nature Methods 13(8), 627 (2016).

[2] Dean-Ben, X. L., Gottshalk, S., McLarney, B., Shoham, S., Razansky D., "Advanced optoacoustic methods for multiscale imaging of in vivo dynamics," Chemical Society Reviews 46(8), 2158-2198 (2017).

[3] Aguirre, J., Schwarz, M., Garzorz, N., Omar, M., Buehler A., Eyerich K., Ntziachristos V., "Precision assessment of label-free psoriasis biomarkers with ultra-broadband optoacoustic mesoscopy," Nature Biomedical Engineering 1(5): 1-8 (2017).

[4] Park, S., Lee, C., Kim, J., Kim, C., "Acoustic resolution photoacoustic microscopy," Biomedical Engineering Letters 4(3): 213-222 (2014).

[5] Li, L., Zhu, L., Ma, C., Lin, L., Yao, J., Wang, L., Maslov, K., Zhang, R., Chen, W., Shi, J., Wang, L. V., "Single-impulse panoramic photoacoustic computed tomography of small-animal whole-body dynamics at high spatiotemporal resolution," Nature Biomedical Engineering 1(5): 1-11 (2017).

[6] Mercep, E., Herraiz, J. L., Dean-Ben, X. L., Razansky, D., "Transmission-reflection optoacoustic ultrasound (TROPUS) computed tomography of small animals," Light: Science & Applications 8(1): 1-12 (2019).

[7] Ermolayev, V., Dean-Ben, X. L., Mandal, S., Ntziachristos, V., Razansky, D., "Simultaneous visualization of tumour oxygenation, neovascularization and contrast agent perfusion by real-time three-dimensional optoacoustic tomography," European Radiology 26(6): 1843-1851 (2016).

[8] Chen, Z., Dean-Ben, X. L., Gottschalk, S., Razansky, D., "Hybrid system for in vivo epifluorescence and 4D optoacoustic imaging," Optics Letters 42(22): 4577-4580 (2017).

[9] Basak, K., Dean-Ben, X. L., Gottschalk, S., Reiss, M., Razansky, D., "Non-invasive determination of murine placental and foetal functional parameters with multispectral optoacoustic tomography," Light: Science & Applications 8(1): 1-10 (2019).

[10] Weber, J., Beard, P. C., Bohndiek, S. E., "Contrast agents for molecular photoacoustic imaging," Nature Methods 13(8): 639-650 (2016).

[11] Dean-Ben, X. L., Ding, L., Razansky, D., "Dynamic particle enhancement in limited-view optoacoustic tomography," Optics Letters 42(4): 827-830 (2017).

[12] Chaigne, T., Arnal, B., Vilov, S., Bossy, E., Katz, O., "Super-resolution photoacoustic imaging via flow-induced absorption fluctuations," Optica 4(11): 1397-1404 (2017).

[13] Kim., J., Kim, J. Y., Jeon, S., Baik, J. W., Cho, S. H., Kim, C., "Super-resolution localization photoacoustic microscopy using intrinsic red blood cells as contrast absorbers," Light: Science & Applications 8(1): 1-11 (2019).

[14] Vilov, S., Arnal, B., Bossy, E., "Overcoming the acoustic diffraction limit in photoacoustic imaging by the localization of flowing absorbers," Optics Letters 42(21): 4379-4382 (2017).

[15] Dean-Ben, X. L., Razansky, D., "Localization optoacoustic tomography," Light: Science & Applications 7(4): 18004 (2018).

[16] Zhang, P., Li, L., Lin, L., Shi, J., Wang, L. V., "In vivo superresolution photoacoustic computed tomography by localization of single dyed droplets," Ligth: Science & Applications 8(1): 1-9 (2019).

[17] Degtyaruk, O., McLarney, B., Dean-Ben, X. L., Shoham, S., Razansky, D., "Optoacoustic calcium imaging of deep brain activity in an intracardially perfused mouse brain model," Photonics 6(2): 67 (2019).

[18] McLarney, B., Rebling, J., Chen, Z., Dean-Ben, X. L., Gottschalk, S., Razansky, D., "Uniform light delivery in volumetric optoacoustic tomography," Journal of Biophotonics 12(6): e201800387 (2019).

[19] Ozbek, A., Dean-Ben, X. L., Razansky, D., "Realtime parallel back-projection algorithm for three-dimensional optoacoustic imaging devices," European Conference on Biomedical Optics, 88000I (2013).